Perivenous application of fibrin glue reduces early injury to the human saphenous vein graft wall in an ex vivo model

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Abstract

Objectives: From animal and clinical studies it is known that prevention of ‘overdistention’ of vein grafts by using extravascular support ameliorates the arterialization process in vein grafts with subsequent more favorable patency. The most ideal support is a biodegradable, porous, elastic graft (Biomaterials, 15 (1994) 83). However, a specific graft meeting these criteria is not available yet. Fibrin glue on the other hand, although used for other purposes in cardiac surgery, theoretically meets the criteria for ideal extravascular support. In this ex vivo study, we evaluated the possible beneficial effect of perivenous application of fibrin glue. Methods: Segments of human vein graft obtained during CABG procedures in 14 consecutive patients were placed in a side loop of the extracorporeal perfusion circuit. In this way the study vein grafts did meet identical circumstances as the vein grafts implanted. Perfusion in the loop was started with a flow just enough to counteract the collapse of the vein, usually about 8 mmHg, and alternately around the segments fibrin glue was applied or no perivenous support was administered as control. After 1 min of solidification, perfusion was started with a pressure of about 60 mm Hg (non-pulsatile flow). Perfusion was maintained for 60 min, after which the grafts were collected for light microscopic and electron microscopic assessment. Results: Light microscopy and electron microscopy showed remarkable attenuation of endothelial cell loss and less injury of smooth muscle cells of the circular muscle layer of the media in the fibrin glue supported vein grafts compared to the non-supported group. Conclusion: Fibrin glue is able to accomplish adequate external vein graft support, preventing overdistention, in an ex vivo model. This provides a basis for clinical application. Further investigation is necessary to evaluate long-term effects. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Vein graft; Perivenous support; Endothelium; Smooth muscle cell; Fibrin glue

1. Introduction

The patency rates of human vein grafts following coronary artery bypass grafting (CABG) are generally less favorable than those of selected arterial grafts. Mammary arteries [2], the right gastro-epiploic artery [3], and the radial artery [4], are commonly used. Vein grafts, however, still are used in most patients, although they show less favorable patency rates. Identification of factors promoting occlusion indicated that ‘overdistention’ of the vein graft due to the higher arterial pressure, is an important factor in the damage of the vein graft wall [5]. In animal and human in vitro experiments, and small clinical trials the beneficial effect of applying microporous, elastomeric, biodegradable perivenous support has been shown [1,6–11]. However, easy to apply perivenous support is not available clinically. Every external application in the form of a graft or a stent implicates handling and instrumentation of the vein graft with subsequent risk of injury to the vein graft wall. Besides, technical limitations might have consequences for side to side anastomoses. The Biocompound® graft, which is used clinically for the purpose of external vein graft support in varicose
vein grafts, has a rather digressive means of application [11].

A favorable alternative may be an external graft support in the form of a spray, which can be applied immediately after completion of the bypass graft anastomoses and before exposure to high arterial pressure. On theoretical grounds fibrin glue might give this support [12,13]. As fibrin glue is frequently used in cardiac surgery and is directly available and easy to apply, we decided to evaluate whether fibrin glue can be used as perivenous support. Commercially available fibrin glue (Tissucol®, Hemaseel APR™) is a biological two-component fibrin sealant which is used to achieve hemostasis, to seal leakages, to glue tissue or to support sutures. The product is manufactured from pooled plasma of selected donors. The sealant components, consisting of protein concentrate with fibrinogen as major component mixed with aprotinin and thrombin mixed with CaCl$_2$, is available in a so-called duploject system which allows for single-handed application, thorough mixing and thin-layer spray application. The sealant components are stored frozen and thawed shortly before application. Fibrin glue is elastic, biodegradable and permeable, and does not impede ingrowth of vasa vasaorum. An additional advantage of fibrin glue could be the possibility to add certain proliferation inhibitors, which are for example, known to inhibit intimal hyperplasia [14,15]. We, therefore, investigated in this ex vivo study whether fibrin glue application as perivenous support prevents the immediate injury to the vein graft wall when exposed to the arterial pressure.

2. Materials and methods

Patients were included in the study after informed consent. The study was approved by the local ethical committee. Anesthesia and cardiopulmonary bypass (CPB) were performed according to the routine protocol.

The ex vivo model is a modification of the in vitro model described earlier [16] and consists of a small roller pump (Stöckert) and a vein irrigation set (Bentley laboratories Europe, Uden, the Netherlands), connected to the extra corporeal circuit with an inflow cannula attached to the arterial filter of the arterial line, and an outflow cannula connected to the cardiotomy reservoir (Fig. 1). The pressure in the ex vivo system is equalized with the blood pressure of the patients by varying the distal occlusion and by adjusting the flow. After start of the extracorporeal bypass the study saphenous vein graft, harvested with the no touch technique, and with a length of about 3 cm, was mounted in the ex vivo perfusion system. Consequently, the study vein grafts were perfused with autologous blood. At no point in the harvesting procedure distention was allowed before perfusion in the perfusion system and no local spasmylytic agents were applied. Before pressurizing the vein graft segment, but after start of the perfusion with a pressure just enough to counteract the collapse of the vein (usually about 8 mmHg), alternately in consecutive patients, fibrin glue (Tissucol®, Baxter, Hyland Immuno Division) was administered as a spray according to the directions of the manufacturer, or no perivenous support was applied. After the application of fibrin glue and after 1 min of curing, the intraluminal pressure was allowed to approximate the arterial pressure of the patient. Flow in the vein grafts was about 100 ml/min. The study vein grafts were placed in the pericardium during perfusion. In this way the vein graft segments were exposed to exactly the same conditions as the vein grafts implanted in the patients. After 60 min the study vein graft perfusion was terminated and the vein graft was collected for light and electron microscopy. All specimens for morphologic analysis were taken from the central part of the study vein graft segments to exclude trauma caused by ligation or turbulence at the canula tips.

2.1. Immunohistochemistry

The vein graft segments were fixed in formaldehyde and embedded in low melting point paraffin wax. Transverse sections of formaldehyde embedded tissue samples were cut at 5 µm and were stained with hematoxylin–eosin and with Elastica Von Giesson.

For immunohistochemistry endogenous peroxidase activity was blocked by 0.3% (v/v) H$_2$O$_2$ in methanol. After preincubation with normal rabbit serum (Dakopatts A/S, Glostrup, Denmark) during 15 min (1:50), the slides were incubated for 60 min with the antibody CD31 (1:40) (Dakopatts A/S, Glostrup, Denmark) and CD34 (1:25) (Becton and Dickinson).

2.2. Electron microscopy

The vein wall segments were fixed in 2% (v/v) glutaraldehyde for 30 min and 1.5% (w/v) osmiumtetroxide for 10 min, dehydrated with acetone and embedded in Epon 812. Ultra-thin sections were collected on 300-mesh Formvar-coated Nickel-grids. The sections were contrasted with uranyl acetate and lead citrate and examined in a Jeol 1200 EX electron microscope.

2.3. Histologic analysis

The endothelial layer was assessed by two investigators (HWMN and WS) in hematoxylin–eosin, CD31, and CD34 stained sections. The circumference of the vein graft segments were divided in 12 parts and the percentage of endothelium left in each part was determined. This resulted in a mean percentage of covering endothelium, which was translated in a score per vein graft according to the numeric grading system of Griffith et al. [17] to score the uniformity, continuity, and integrity of the histologic structures: 0 (intact, no disruption), 1 (<10%), 2 (10–25%), 3 (25–50%), and 4 (<50%).

The internal lamina, medial smooth muscle and connective tissue were assessed on the electron microscopical
pictures of two representative transmural samples of each study vein graft by the same investigators according to the criteria of the same scoring system. Due to the attachment of fibrin glue blinding was not possible.

2.4. Statistics

Statistical analysis of the histologic scores was carried out using the Kruskal–Wallis test for non-parametric data. All data were analyzed using the SPPS 9.0.1 software. A probability value of $P \leq 0.05$ was considered statistically significant upon two-tailed testing.

3. Results

During the experiments apparent distention was noticed in the non-supported vein graft segments. The vein graft segments with perivenous fibrin glue support, however, showed no distention during the perfusion (not shown).

Light microscopy with CD34 as an endothelial marker shows complete endothelial cell loss in the unsupported vein grafts (Fig. 2a), whereas the supported vein grafts show an intact endothelial layer (Fig. 2b).

Electron microscopical examination in the non-supported group showed an extensive destruction of the basal lamina and loss of endothelium (Fig. 3a). All supported grafts had a normal and intact basal lamina and an intact endothelial cell lining (Fig. 3b). In the media of the non-supported group, the organoid arrangement of the collagen fibers was lost and edema was found, whereas the media showed no disarrangement of collagen fibers and no edema in the supported group. Extensive vacuolisation was found in the smooth muscle cells of the circular layer in the non-supported group, which represents important injury (Fig. 4a). In the
supported group, the smooth muscle cells of both the longitudinal and circular layer were intact but a slight degree of vacuolisation in the circular muscle layer was present (Fig. 4b).

The results of the histologic evaluation are given in Table 1. The differences in the injury score concerning the endothelial layer, the basal lamina, the connective tissue, and the circular muscle layer are significant. No significant difference was found for the longitudinal muscle layer.

4. Discussion

This morphological study demonstrates that perivenous support of human saphenous vein graft segments with fibrin glue in an ex vivo model can attenuate the severe injury encountered in the non-supported vein graft exposed to arterial pressure. The findings in the non-supported vein grafts in our model are in concurrence with the post-mortem findings in saphenous vein coronary artery bypass grafts by Kockx et al. [18] who show almost complete deendothelialization within 24 h after transplantation, and severe damage in the circular smooth muscle cell layer of the media of the implanted graft.

The protective effect of fibrin glue support on human saphenous vein graft segments is comparable with the effect of a perivenous PTFE support as we have found earlier in the perfused vein model [16]. In the underlying study we used CD34 as an endothelial marker, while we used CD31 earlier. CD34 is a more specific marker for endothelium as compared to CD31, which is also present on platelets and leukocytes and may, therefore, give a false positive result. A false positive impression was indeed caused by platelet coverage of damaged vessels as demonstrated in pressure exposed veins by electron microscopy [16].

The easy to apply, sprayable fibrin glue brings the external vein graft support within clinical reach, but the question whether the support lasts sufficiently long to obtain adequate...
protection and arterialization of the graft still remains to be answered. In this respect it is of interest to note that the fibrin glue as a perivenous support has the potential advantage that it provides the possibility to incorporate protease inhibitors in the gel, as well as other stimulatory and inhibitory agents that can modulate vein graft remodeling and intimal hyperplasia.

Fibrin is not an inert molecule. Fibrin and fibrin degradation products are known to stimulate cell migration and invasion [19]. Application of fibrin glue around the venous graft may, therefore, promote migration of smooth muscle and fibroblastoid cells or other cell types into it. This may promote outward remodeling or adhesion formation between the vessel and the underlying tissue. An intact endothelial layer prevents thrombus formation inside the vessel, and thus limits smooth muscle cell migration into the intima [19]. In this way next to the protective effect of the fibrin glue support, resulting in preservation of the endothelial layer and attenuation of smooth muscle cell injury, the modulating effect of fibrin on smooth muscle cell migration might further decrease the stimulus for intimal hyperplasia and promote the arterialization process of the vein graft wall.

The major uncertainty of the application of external fibrin glue support of venous bypass grafts is the in vivo stability of the fibrin matrix. If the fibrin matrix would lyse rapidly, the venous graft will slowly distend and the start of venous damage and neointima formation would only be delayed by a couple of days. However, if the remodeling of the fibrin matrix proceeds more slowly, adaptive enforcement of the circular muscle layer of the venous graft may occur without luminal narrowing. Additional experiments in vivo have to provide information regarding the stability of the perivenous fibrin coat when used as perivenous support.

Another concern may be the source of fibrin glue. The commercially available fibrin glue we used in our experiments is manufactured from pooled plasma of selected donors. No procedure has been shown to completely eliminate the risk of viral infectivity from derivatives of human plasma. Although there is some general concern of viral disease transmission, for more than 20 years of product

Table 1
Effect of use of fibrin glue on saphenous vein histology

<table>
<thead>
<tr>
<th></th>
<th>Fibrin glue support (n = 7)</th>
<th>No fibrin glue support (n = 7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of endothelium</td>
<td>0.14 ± 0.38</td>
<td>4.0 ± 0.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Disruption of basal lamina</td>
<td>0.0 ± 0.0</td>
<td>3.7 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Disorderly arrangement of collagen</td>
<td>1.0 ± 0.58</td>
<td>3.9 ± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>SMC injury in longitudinal muscle layer</td>
<td>0.57 ± 0.53</td>
<td>2.0 ± 0.0</td>
<td>0.001</td>
</tr>
<tr>
<td>SMC injury in circular muscle layer</td>
<td>2.0 ± 0.0</td>
<td>3.9 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* The graded score represents the percent disruption expressed as mean ± SD and was scored according to the scale described by Griffith et al. [17]: 0 (no disruption), 1 (<10% disruption), 2 (10–25% disruption), 3 (25–50% disruption), and 4 (>50% disruption).

* Statistical significance determined using Kruskal–Wallis (rank-sum) test for non-parametric data.

* Graded score represents the percent disruption expressed as mean ± SD and was scored according to the scale: 0 (no disruption), 1 (<10%), 2 (10–25%), 3 (25–50%), and 4 (>50%).
use to our knowledge there was no single report of seroconversion for Human Immuno-deficiency Virus, Hepatitis B Virus and Hepatitis C Virus.

In conclusion, external vein graft support with fibrin glue might offer adequate protection from early saphenous vein graft injury and promote remodeling of the vein graft in the direction of arterial wall characteristics.

References


